ORIGINAL ARTICLE

Clinical utility of a dipstick assay in patients with brucellosis: correlation with the period of evolution of the disease

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To examine the clinical utility of a dipstick assay for the detection of *Brucella*-specific IgM antibodies, and the correlation with the evolution of the disease. Twenty-six patients who were admitted to the General Hospital of Albacete (Spain) over a 2-year period and diagnosed with brucellosis were included in the study. One hundred and twenty-five serum samples collected at the time of diagnosis and at intervals during and after treatment were tested by the Coombs test, the standard seroagglutination test (SAT), the SAT in the presence of dithiothreitol (DTT-SAT), and a dipstick assay for the detection of *Brucella*-specific immunoglobulin M (IgM) antibodies. The sensitivity of the dipstick assay at the moment of the diagnosis was similar to that of the SAT (62% and 73%, respectively), somewhat higher than that of the DTT-SAT (50%), and lower than that of the Coombs test (100%). Patients with a negative dipstick test at the moment of diagnosis displayed a period of evolution of the disease longer than that of the dipstick-positive patients. After the beginning of therapy, the detection rate of the dipstick assay decreased faster than those of the SAT, the DTT-SAT, and the Coombs test. Thirty days after the start of therapy, the detection rate of the dipstick assay had decreased to 7%, whereas that of the SAT and DTT-SAT was 46%, and that of the Coombs test was still 92%. The dipstick assay could be used as a rapid diagnostic test for patients in the early stages of illness. Patients with a long period of illness will probably have a negative dipstick test, and could be diagnosed with the aid of the Coombs test and classical clinical findings.

Keywords Human brucellosis, serology, standard serum agglutination test, Coombs, dipstick assay, immunoglobulin M antibodies, acute disease

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INTRODUCTION

Human brucellosis is a multisystem disease that may present with a broad spectrum of clinical and serologic manifestations [1]. The isolation of the microorganism from blood samples is the confirmatory test for the disease. However, the sensitivity of this technique varies depending on the stage of the disease, and special conditions are required to work with the pathogen. When the culture is negative, the classical serologic tests (the standard seroagglutination test (SAT) and the Coombs test) are essential for diagnosis of the disease [2,3]. These tests detect antibodies against the lypopolysaccharide (LPS) of *Brucella* [4]. The SAT detects immunoglobulin M (IgM), IgG and IgA agglutination antibodies. The SAT performed in the presence of a reducing agent such as dithiothreitol (DTT-SAT) is used to detect IgM agglutinating antibodies, which are present early in the infection. The Coombs test detects IgG and IgA class antibodies, which persist long after the agglutinating antibodies have declined, and often remain long after the patient is cured. The test cannot be used

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for differentiating between stages of the disease. Patients with relapse and chronic brucellosis have levels of IgG antibodies that are detected by the Coombs test. The SAT and the Coombs test may give false-positive results when used for testing patients in endemic countries, due to the presence of persisting antibodies [5,6].

Rapid serologic tests that provide immediate information at the time of clinical diagnosis are desirable [1]. The most important is the Rose Bengal test, an agglutination test that provides results in 2–3 min. It is very useful as the first screening test. However, this technique has two disadvantages: first, cross-reactions with bacteria related to the genus *Brucella*; and second, positive results in exposed populations, and in patients after they are cured [3].

During the last few years, two new techniques have been developed for the diagnosis of human brucellosis, Brucellacapt and dipstick. Brucellacapt is an agglutination test that determines antibodies of all classes specific to *Brucella* [7,8], with the advantage of the results being obtained in 24 h. The dipstick assay is a rapid test that detects *Brucella*-specific IgM antibodies [9]. The technique provides a quick result, is easy to perform, and does not require trained personnel. When Smits et al. applied it to a group of laboratory-confirmed cases and controls from five different countries, a sensitivity of 89% and a specificity of 98.6% were demonstrated for samples collected early in the evolution of the disease [9]. However, a further prospective study is needed to examine the clinical utility of the assay and the correlation with the stage of disease.

MATERIALS AND METHODS

Patients and clinical specimens

Twenty-six patients who were admitted to the General Hospital of Albacete (Spain) over a 2-year period were included in the study. The diagnostic criteria were: (1) the isolation of *Brucella* species from blood or other body fluid or tissue specimens; and (2) the presence of compatible clinical findings (fever, sweats, arthralgias, headache, weight lost, hepatomegaly, splenomegaly, or signs of focal disease) with the demonstration of specific antibodies to *Brucella* at significant titers. Significant titers were considered to be a SAT titer $\geq 1:160$, and a Coombs test titer $\geq 1:320$. The result of the DTT-SAT was considered to be significant when a $\geq$2-fold decrease in titer compared with the SAT was obtained.

The patients were given different antibiotic regimens, because they were involved in several trials [1]. Routine blood cultures and serologic and clinical evaluations were performed at the time of clinical diagnosis and at intervals during and after treatment. In total, 125 serum samples were collected. The samples were stratified into samples collected at the time of the clinical diagnosis ($n = 26$), during the first ($n = 14$) and second ($n = 13$) weeks after the beginning of treatment, during the second ($n = 19$) and third ($n = 15$) months, between the third and sixth months ($n = 19$), and after the sixth month ($n = 19$).

Serologic methods

The SAT, the DTT-SAT and the Coombs test were performed as described elsewhere [10]. The dipstick assay consists of a strip of nitrocellulose-containing specific antigen applied in a distinct line, and a non-enzymatic detection reagent. The antigen consists of an LPS fraction prepared by heat extraction from a washed culture of *B. abortus* strain 1119-2. The detection reagent consists of a monoclonal antihuman IgM antibody conjugated to Palanyl red [11,12]. The dipstick assay was performed by the incubation of a wetted dipstick for 3 h in 250 μL of detection reagent mixed with 5 μL of a serum sample. At the end of the incubation period, the strip was rinsed with tap water to remove the excess detection reagent, and dried at room temperature. The staining intensity was rated 1+ to 4+ by comparison with a colored reference strip. The dipstick assay was considered positive when a staining intensity $\geq$1+ was observed.

Statistical analysis

Values of the continuous variables are expressed as medians and ranges. *P*-values, calculated by Epi Info, version 6, were considered to indicate statistical significance when they were less than 0.05 by two-sided tests [13].

RESULTS

Characteristics of the patients

Twenty-six patients were included in the study; 20 were male and six female (Table 1). The ages of the

Table 1 Diagnostic criteria, duration of illness and serologic findings at diagnosis

Patient no.	Diagnostic criteria	Duration of illness at diagnosis (days)	Assay			
			Dipstick	SAT	DTT-SAT	Coombs
1	Synovial fluid +	30	0[b]	80[c]	40	640
2	Arthritis in shoulders; CF[a]	20	0	160	40	2560
3	CF	100	0	640	160	10 240
4	Arthritis in shoulders and knees	160	0	80	40	2560
5	CF	365	0	40	<40	320
6	Arthritis in knees	1095	0	<40	<40	10 240
7	CF	20	0	80	<40	320
8	Blood culture +	30	0	80	40	1280
9	CF	365	0	40	<40	320
10	CF	15	0	640	320	20 480
11	Blood culture +	45	1	320	160	640
12	Blood culture +	3	1	160	40	2560
13	CF	50	1	640	160	2560
14	Blood culture +	20	1	160	40	1280
15	Sacroiliitis	15	1	160	80	2560
16	Blood culture +	3	2	2560	40	2560
17	CF	45	2	1280	640	10 240
18	Sacroiliitis	20	2	320	160	2560
19	CF	1095	2	160	40	640
20	Subclinical brucellosis[d]	5	3	640	40	1280
21	CF	20	3	1280	640	2560
22	Blood culture +	20	3	320	40	650
23	CF	390	3	320	40	2560
24	Blood culture +	18	4	1280	80	2560
25	CF	12	4	40 960	2560	40 960
26	CF	15	4	1280	<40	1280

[a]CF, clinical findings (fever, sweats, arthralgias, hepatomegaly, headache, weight lost, splenomegaly, or signs of focal disease). [b]Staining intensity. [c]Reciprocal titer. [d]This patient had a history of ingestion of unpasteurized dairy products, and presented malaise and weakness.

26 patients ranged from 17 to 68 years (median: 37.5 years). *B. melitensis* was isolated from seven blood cultures and one synovial fluid culture. The remaining cases, with negative culture results, were diagnosed on the basis of serologic criteria and characteristic clinical findings. The duration of symptoms before admission ranged from 3 days to 3 years (median: 20 days). After treatment, no patient suffered from relapse or reinfection.

Comparison of serologic tests at diagnosis

The sensitivity of the dipstick assay at the time of diagnosis of the disease was 62%, similar to that of the SAT (73%; $P = 0.37$), and lower than that of the Coombs test (100%; $P = 0.0004$). Although the presence of a reducing agent (DTT) decreased the sensitivity of the SAT to 50%, there was no statistical difference with the dipstick assay ($P = 0.26$).

Ten patients (38%) were dipstick negative at the moment of diagnosis (Table 1). Three of them were SAT positive, of whom two were DTT-SAT positive. All dipstick-negative patients were Coombs positive (Table 1). These ten patients had had symptoms before the diagnosis of the disease for more than 15 days (median: 65 days); three had chronic disease (>1 year), and three presented with complications (arthritis).

The samples from 16 patients who had positive results at the time of the diagnosis were also SAT positive. However, the samples from five of these patients were DTT-SAT negative. The period of time with symptoms before diagnosis was less than 50 days for most patients (median: 20 days),

but two had chronic infection and two presented with complications (sacroiliitis).

Response to treatment

After the start of treatment, the positivity of the dipstick assay decreased faster than that of the SAT, the DTT-SAT, and the Coombs test (Table 2). Thirty days after the beginning of therapy, the detection rate of the dipstick assay had decreased to 7%. The detection rate of the SAT and DTT-SAT was 46% at this time point ($P = 0.057$), and that of the Coombs test was still 92% ($P = 0.0004$). Six months after therapy, a negative result for all patients was obtained only in the dipstick assay. The SAT and DTT-SAT were still positive in 11% of the patients, and the Coombs test in 63%.

Correlation between the dipstick test and the DTT-SAT

A negative result in the dipstick assay was obtained for 97 samples. A weak (1+) positive result was obtained for 10 samples, and a moderate (2+) to strong (4+) positive result for 18 samples. Concordant results for the dipstick assay and the DTT-SAT were obtained for 18 samples that gave a positive result in the two tests and for 85 samples that gave a negative result. Discrepant results for the two tests were obtained for 10 dipstick-positive samples and 12 dipstick-negative samples. The agreement between the two tests was 82%. Most discrepant results gave either a weak (1+) staining intensity in the dipstick assay or a two-fold reduction in titer in the DTT-SAT. However, a moderate to strong staining intensity of the dipstick correlated with a >two-fold reduction in titer in the DTT-SAT in most cases. The correlation coefficient between the staining intensity observed in the dipstick assay and the degree of reduction in titer in the DTT-SAT was 0.68.

Table 2 Positivity of the dipstick assay, the SAT, the DTT-SAT and the Coombs test in relation to time after start of treatment ($n = 26$)

	Positivity (%)			
Time (n)[a]	Dipstick	SAT	DTT-SAT	Coombs
0 (26)	62	73	50	100
1–2 weeks (14)	50	86	50	100
3–4 weeks (13)	7	46	46	92
2 months (19)	16	47	31	100
3 months (15)	0	28	21	86
3–6 months (19)	0	21	15	68
>6 months (19)	0	10	10	63

[a]Number of serum samples.

DISCUSSION

The diagnosis of human brucellosis requires isolation of the bacteria or confirmation through serologic tests. However, culture sampling sensitivity is often low, depending on the disease stage, *Brucella* species, culture medium, quantity of circulating bacteria, and blood culture technique employed [14,15]. Patients with long periods between onset of disease and diagnosis could give false-negative results. This would explain the low sensitivity of blood cultures in this group of patients (30%).

On the other hand, the results of most standard serologic tests can be obtained only after 24 h (SAT, Coombs), and trained personnel are necessary to perform these tests. Therefore, the availability of a serologic test providing immediate information at the time of clinical diagnosis would be advantageous. The dipstick assay is a rapid and simple assay that detects IgM anti-*Brucella* antibodies within 3 h [9]. In the study carried out by Smits et al. [9], a sensitivity of 89% was calculated for the dipstick assay for samples collected during the first 2 months after the onset of disease. The sensitivities of the SAT, DTT-SAT and Coombs test were 79.5%, 67.1% and 39.7%, respectively. These data were very promising, but it was necessary to evaluate the use of the test with regard to the stage of disease. Consequently, we decided to assess it in a group of 26 patients diagnosed with brucellosis. The sensitivity of the dipstick assay for this group of patients at the moment of the diagnosis was similar to that of the SAT (62% and 73%, respectively), higher than that of the DTT-SAT (50%), and lower than that of the Coombs test (100%). The period of time from the onset of disease hospitalization varies from days to months, or even years. The detection of specific IgM antibodies is regarded as a sign of acute brucellosis. In those patients with a long history of illness, the level of specific IgM antibodies is decreased, decreasing the sensitivity of the SAT and DTT-SAT [16]. The ten patients with a negative result in the dipstick assay presented at a later stage (median: 65 days) than the 16 patients with a

positive result in the dipstick assay (median: 20 days), explaining the relatively low sensitivity for the total group. The percentage of patients with chronic brucellosis and the percentage of patients with complications were higher in the group of patients with a negative result in the dipstick assay.

The Coombs test was the only test that gave a positive result at the time of diagnosis for all patients. The Coombs test, in contrast to the dipstick assay, remained positive for the majority of patients at the end of therapy, and stayed positive for more than 3 months thereafter. The dipstick assay became negative rapidly after the beginning of therapy, with a positive result for only 7% of the patients at 30 days, and with a negative result in all patients after 3 months of treatment. The SAT and DTT-SAT also remained positive for a longer period and in a higher percentage of the patients after the end of therapy. The occurrence of persistent high titers, even when the patient is cured, is considered one of the main problems in the serodiagnosis of brucellosis [5].

In conclusion, the dipstick assay provides rapid diagnosis of patients with brucellosis in the early stages of the disease. Specific IgM antibodies that are detected in the dipstick assay are present in the serum of patients during the early stages of the disease. Patients with a long period of evolution will probably have a negative dipstick test, but can be diagnosed with the aid of the Coombs test and classical clinical findings.

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